Rapid Papers

(Pages 877–887)

Biochem. J. (1979) **182**, 877–879
Printed in Great Britain

The Subcellular Distribution of Alanine–Glyoxylate Aminotransferase and Serine–Pyruvate Aminotransferase in Dog Liver

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(*Received* 29 *May* 1979)

The subcellular distributions of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase in the particulate fraction of dog liver were examined by centrifugation in a sucrose density gradient. Most of both enzyme activities in the particulate fraction were localized in the mitochondria, but not in the peroxisomes.

Alanine–glyoxylate aminotransferase (EC 2.6.1.44) and serine–pyruvate aminotransferase (EC 2.6.1.51) are distributed in livers of a wide range of animal species (Rowsell *et al.*, 1969, 1972*a*; Noguchi *et al.*, 1978*a*). In the rat, bold enzyme activities are increased by the injection of glucagon, possibly by a cyclic AMP-dependent mechanism (Rowsell *et al.*, 1969; Snell & Walker, 1972, 1974; Noguchi *et al.*, 1977, 1978*a*,*b*). We have previously described that these glucagon-inducible enzyme activities of rat and mouse liver are associated with the same protein. Alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities of dog liver are also properties of a single protein, and similar findings have been made with cat liver (Noguchi *et al.*, 1978*a*). Some 50–55% of the particulate serine–pyruvate aminotransferase activity is localized in the peroxisomes and the remainder in the mitochondria, and most alanine–glyoxylate aminotransferase activity is localized in the mitochondria, with some activity in the peroxisomes in rat liver (Noguchi *et al.*, 1978*b*).

Noguchi & Takada (1978) claimed that serine–pyruvate aminotransferase is located only in the peroxisomes and in the soluble fraction in human liver.

For a proper consideration of the roles of these enzymes in cellular metabolism, ample and reliable knowledge of their subcellular location is required. The present paper describes that, in dog liver, alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase are mainly present in the mitochondria; the subcellular localization is different from that of the enzymes of rat and human liver.

Experimental

Materials

Reagents. All materials used were obtained as stated previously (Noguchi *et al.*, 1976*a*,*b*, 1978*a*). Digitonin was from E. Merck, Darmstadt, Germany. Nembutal (sodium pentobarbital) was from Abbott Laboratories, North Chicago, IL, U.S.A. *p*-Nitrophenyl phosphate (disodium salt) was from Wako Pure Chemical Industries, Osaka, Japan.

Animals. Male dogs of mixed breed were anaesthetized by the injection of 250mg of Nembutal. The liver was perfused with ice-cold 0.9% NaCl, before samples were taken for homogenization.

Methods

Preparation of subcellular fractions. All procedures were carried out at 0–4°C. Liver was minced and homogenized in 0.25M-sucrose (in water) by one stroke of a Teflon pestle in a Potter–Elvehjem homogenizer at 700rev./min. The homogenate (10%, w/v) was centrifuged at 10000*g* for 30min. The resulting pellet was homogenized with 0.25M-sucrose and filtered through one layer of coarse cheesecloth, and centrifuged as described above. The supernatant was discarded. The resulting pellet was suspended in 0.25M-sucrose at a final concentration of 0.2g wet wt. of the original tissue/ml. Then 5ml of the suspension was mixed thoroughly with 1.75ml of 1% (w/v) digitonin (dissolved in 0.25M-sucrose), and 5ml of the resulting suspension was immediately laid on a 45ml linear sucrose gradient (27–60%, w/w) (in water). The rotor (Hitachi RPS 25–2) was accelerated to 10000rev./min (12000*g*) and maintained at this speed for 10min, and then accelerated further to 25000rev./min (74000*g*) and maintained at this speed for 2.5h, in a Hitachi 65P ultracentrifuge (Hitachi, Tokyo, Japan) at 4°C. Fractions (30 drops) were collected by pumping out from the bottom of the centrifuge tube with a Mitsumi Gradienter SJ-1300 UD (Mitsumi Scientific Industry Co., Tokyo, Japan).

Determination of enzyme activities. L-Alanine–glyoxylate aminotransferase and L-serine–pyruvate aminotransferase were assayed as described by

Rowsell *et al.* (1972*c*) and Snell & Walker (1974), respectively. Catalase (EC 1.11.1.6) (Sinha, 1972), glutamate dehydrogenase (EC 1.4.1.2) (Beaufay *et al.*, 1959) lactate dehydrogenase (EC 1.1.1.27) (Kornberg, 1955) and acid phosphatase (EC 3.1.3.2) (Tani & Ogata, 1970) were assayed as described in the cited references.

A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of product or a decrease in substrate of 1 μmol/min, at 37°C for aminotransferase and acid phosphatase and at 20°C for catalase, glutamate dehydrogenase and lactate dehydrogenase.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and Discussion

Some 70–80% of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities of dog liver homogenate were localized in the particulate fraction (10000*g* pellet) and the remainder were in the supernatant fraction (10000*g* supernatant). Recoveries of glutamate dehydrogenase (the mitochondrial-matrix marker) activity were 74% and 36% of the homogenate activities in the particulate and the supernatant fractions respectively. In spite of the use of the same differential fractionation, dog mitochondria were more disrupted than those of the rat (Rowsell *et al.*, 1972*b*; Snell, 1975). This may be due to the difference between their characters. Activity of lactate dehydrogenase, a cytosol marker, was completely recovered in the supernatant fraction.

A typical sedimentation in a sucrose density gradient from the particulate fraction treated with digitonin is shown in Fig. 1. The marker enzymes of the peroxisomal and mitochondrial fractions were almost completely separated; the peroxisomal marker, catalase, was retained in the upper zone of the sucrose gradient designated as the soluble fraction, and the mitochondrial marker, glutamate dehydrogenase, was sedimented at densities of 1.27, 1.25, 1.23 and 1.20 g/ml at 20°C and some in the soluble fraction.

A close correspondence between the distribution profiles of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities and that for glutamate dehydrogenase activity was apparent.

Almost all the peroxisomes were ruptured by the digitonin treatment, and the peroxisomal fraction contained only very low alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities. Furthermore, sedimentation profiles of the aminotransferases in the soluble fraction clearly differed from that of catalase, but coincided with that of glutamate dehydrogenase.

Both aminotransferase activities showed different distribution profiles from that of acid phosphatase activity, the lysosomal marker.

The results obtained in this experiment indicate that alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities in the particulate fraction of dog liver are only localized in the mitochondria, but not in the peroxisomal fraction. The localization of these enzymes differs from that of rat liver and human liver enzymes (Noguchi *et al.*, 1978*b*; Noguchi & Takada, 1978).

From the results of sucrose-density-gradient centrifugation, it is concluded that both aminotransferase activities observed in the supernatant fraction are likely to be the result of mitochondrial leakage.

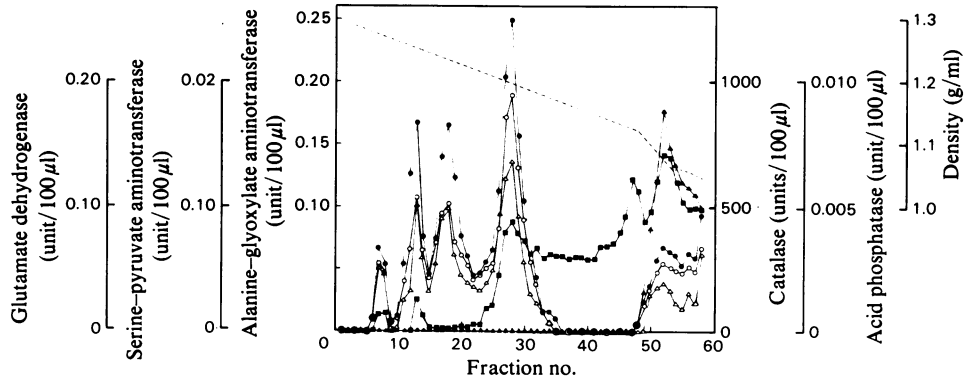

Fig. 1. *Subcellular distribution of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase in dog liver*
Preparation of the particulate fraction, treatment with digitonin and centrifugation in the sucrose density gradient were described in the text. Alanine–glyoxylate aminotransferase (●), serine–pyruvate aminotransferase (○), catalase (▲), glutamate dehydrogenase (△), acid phosphatase (■) activities and densities at 20°C (----).

The separation pattern of mitochondria on the sucrose density gradient as shown in Fig. 1 may be due to the following reasons: the membranes of some of the mitochondria were injured by digitonin; highly concentrated sucrose penetrated into the matrix during the centrifugation and resulted in an increase in the density of the partially injured mitochondria. Without digitonin treatment, mitochondria were sedimented at a density of 1.20g/ml and did not separate from peroxisomes.

Cytosolic alanine–2-oxoglutarate aminotransferase (EC 2.6.1.2) is mainly involved in the formation of alanine from pyruvate, whereas the mitochondrial enzyme present only in gluconeogenic tissue is involved in the conversion of alanine into pyruvate (De Rosa & Swick, 1975). Our observations show an exclusively cytosolic location for alanine–2-oxoglutarate aminotransferase in human (N. Kamoda, Y. Minatogawa, M. Nakamura, J. Nakanishi, E. Okuno & R. Kido, unpublished work) and dog liver (M. Nakamura, Y. Minatogawa & R. Kido, unpublished work). Therefore, the conversion of alanine into pyruvate in dog liver mitochondria may be mainly catalysed by the alanine–glyoxylate aminotransferase, suggesting the implication of the enzyme in gluconeogenesis from alanine.

It has been proposed that hydroxypyruvate is an intermediate in the synthesis of glucose from serine (Dickens & Williamson, 1959; Lardy *et al.*, 1969; Sallach *et al.*, 1972; Rowsell *et al.*, 1973; Snell, 1974; Williamson & Ellington, 1975). The pathway is initiated by serine–pyruvate aminotransferase, which catalyses the transamination of serine to form hydroxypyruvate in rat liver (Snell, 1975). In dog, but not in rat, it is not clear how the serine dehydratase pathway is implicated in gluconeogenesis from serine. However, it has been suggested that the role of serine aminotransferase in gluconeogenesis may be considerably greater in dogs than in rats, because of the high activity of the serine–pyruvate aminotransferase of dog liver (Rowsell *et al.*, 1969, 1972*a*).

The present findings suggest that alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase present in dog liver mitochondria participate in gluconeogenesis from alanine and serine, particularly in carnivores.

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